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Dear Dr. Lederberg

You will find here enclosed a MS reporting a few experiments which grew out of your work with Morse. I hope you find it interesting and I want to take advantage of this letter to thank you for all the fun I have had playing with Gal transduction. Someone (Kalckar) mentioned that you had a paper coming out soon in Genetics and (Lazarji) that Morse had done some UV irradiation of λ HFT. Would it be too much to ask you to send me copies of these two papers?

There are quite a few things which I would like to do with the multiplication of Gal$^+$ (or rather its absence) in Gal$^-$ cells. But since you are perhaps engaged in such type of experiments I may stop my work with Gal. ~~and~~ If however you were not intending to do this sort of thing we could get together and decide that I could do these experiments. In that case I would need a few of your Gal$^-$ strains mutations and I would be very grateful if you could send them to me here.

My regards to your wife. When shall we meet at last? Are there any strains of bacteria or phages you could like to have?

Thank you again

Luigi.

# On Gal Transduction by Phage lambda

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Virus transduction has been defined as the phenomenon in which a virus grown on a bacterial host (the donor) transfers to a new host (the acceptor) a fragment of the genetic content of the donor. In the transductions studied previously the phages were able to transduce any genetic marker of the donor, often but not necessarily rendering at the same time the acceptor lysogenic. Recently Morse (1954) and Morse, Lederberg and Lederberg (1956) have described a new type of transduction involving the strain K12 of E. coli and the phage lambda. The novel features of this type of transduction are:

1) The phage lambda carried (as prophage) by the lysogenic strain K12 is able to transduce the marker Gal⁺ (ability to ferment galactose), and this marker only.

2) Only the phages liberated by lysogenic bacteria have transducing ability. In the experiments of Morse et al. the liberation of the phage from the donor was induced by small doses of UV. It is not known whether spontaneously liberated phages or phage induced by other means would show the same ability to transduce.

3) The transduced cells are invariably lysogenic.

To obtain a transducing lysate one induces a culture of a strain of K12 carrying lambda and able to utilize galactose. Upon infection with this lysate of another strain of K12, sensitive to lambda and unable to ferment galactose (Gal⁻), a few cells (about one in $10^6$ or

$10^7$ infected ones) become able to ferment galactose. Most of these transduced and lysogenic strains are unstable with respect to the Gal character: while transmitting the $Gal^+$ property to most of their offspring they occasionally segregate stable $Gal^-$ cells while remaining lysogenic. These transduced cells have further the remarkable property that when they, in turn, are induced they liberate phages able to transduce $Gal^+$ with a very high frequency. Usually at least 50% of the phages are able to transduce. Morse et al. call these phages high frequency transducers (HFT) and the transduced strains heterogenotes" implying that these strains carry the transduced piece" not as a substitution but as an addition to the genome, perhaps an addition to the phage genome.

The ability to ferment galactose has been used as a genetic marker in bacterial crosses and has been shown by recombination (Lederberg and Lederberg, 1953) and by the previously known transduction (Jacob, 1955) to be closely linked to the lysogenic determinant, presumably the prophage lambda.

The experiments of Morse et al. raise an interesting question concerning the processes occurring in the heterogenote bacterium when this bacterium is induced and the HFT phage is produced: does the $Gal^+$ marker, which eventually gets incorporated in the HFT phage, multiply when the phage multiplied and is its multiplication very closely coupled to that of the phage? An answer to this question might be helpful in clarifying the origin of the transduced piece of the bacterial genome in--at least--this type of transduction: does it represent an inclusion into the phage of a piece of the bacterial

genome when the phage matures, or does there exist an intimate tie between the transduced piece and the phage prior to phage multiplication? In this note it is shown that the phage and the transduced piece multiply together.

## Material and Methods

Bacteria and Phages.--The bacterial strains used were two variants of *E. coli* strains, K12. One called C600 (Appleyard, 1954) is $Gal^-$ (stable) and is available both as sensitive strain and as lysogenic strain for the phage lambda defined as wild type by Kaiser (1955). The other was the strain 112 and has been shown by Wollman (1953) to carry the marker $Gal^-$ linked to the prophage lambda.

The colonies formed by C600 $Gal^+$ on EMB galactose (Lederberg, 1950) agar plates are dark red and have a metallic sheen, while 112 $Gal^-$ colonies are pink. The 112 transduced colonies are red but not as dark as those of C600 and they have no metallic sheen.

The phage stocks were mostly obtained by induction of the lysogenic strain C600 or by induction of the transduced 112.

Infection.--In general, infection of the bacteria with the phages proceeded as follows: the bacteria were grown in tryptone broth to a density of 1 to 2 x $10^9$, centrifuged, resuspended in distilled water containing $10^{-2}$ M of $MgSo_4$, and aerated for one hour at 37°C. The bacteria were then diluted to a density of about 2 x $10^8$ and the phage suspension added. In 20 minutes more than 95% of the phages were adsorbed by the bacteria. To observe transduction 0.05 ml. of adequate dilutions of infected $Gal^-$ bacteria were spread on EMB galactose plates.

On these plates 0.1 ml. of anti-lambda serum (K-30) had been previously spread to prevent reinfection of the bacteria by phage grown and liberated on the plates.

Isolation of heterogenote:--To prepare a heterogenote strain, phages are first obtained by induction of C600 $Gal^+$ lysogenic for lambda. The lysate is treated with chloroform to kill uninduced cells and tested for sterility. According to the method indicated by Morse et al. $2 \times 10^9$ cells of 112 $Gal^-$ sensitive are mixed with about $2 \times 10^9$ phages and 0.05 ml. of the mixture is plated on EMB galactose anti-serum agar plates. After 48 hours of incubation about $10^3$ $Gal^+$ colonies appear on the background of $Gal^-$ cells. Twenty of these colonies were picked, suspended in 1 ml. of water and spread on EMB galactose plates. This purification procedure of picking and spreading was repeated three times. After the third spreading each of the twenty isolates still segregated $Gal^-$ colonies and each of the isolates was lysogenic. One of the twenty isolates was selected for further study. When this strain was induced it liberated phages which transduced the $Gal^+$ marker with very high efficiency. This strain is thus a heterogenote.

Preparation of HFT lysate by induction:--To prepare phages with high efficiency of transduction (lambda HFT) cultures of the heterogenote in tryptone broth (to which $10^{-2}$ M of $MgSO_4$ had been added) were made. When they reached a density of about $10^8$ cells per ml. they were centrifuged and resuspended in buffer at a density of about $2 10^8$. They were then irradiated with a dose of ultraviolet light inducing more than 95% of the cells. Broth was added to the buffer to bring the

density to $10^8$ and the suspension was allowed to lyse in aerated tubes at 37°C. and in darkness (to avoid photoreversal of the induction). The lysates which contained about $10^{10}$ phages per ml. (burst size about 100 phages per bacterium) were carefully treated with chloroform in order to kill any surviving bacteria. These lysates will be referred to as primary HFT in contrast to secondary HFT lysates which are obtained from the primary HFT by one cycle growth on a sensitive strain of bacteria.

Phage stocks from one cycle growth.--To prepare phages having grown on sensitive bacteria forone cycle, these bacteria were prepared for adsorption and used at a density of about $2 \times 10^9$. They were usually infected at a multiplicity between 0.1 and 0.5 phage per bacterium. After allowing twenty minutes for adsorption the suspensions were chilled and centrifuged. The pellet was washed three times, the cells resuspended and centrifuged again, whereupon the washing procedure was repeated and finally the cells resuspended and diluted in tryptone broth. The number of non-adsorbed phages was measured as well as the number of infective centers. The suspensions were aerated at 37°C for 50 minutes (latent period about 42 minutes) when chloroform was added in order to lyse (Sechaud and Kellenberger, 1956) most of the infected cells. The burst size was usually about 50 phages per infected cells and the lysates contained a proportion of non adsorbed parental phages which was always less than $10^{-4}$.

Assay of high frequency transduction.--To determine the efficiency of transduction of the phages and to characterize the transduced cells three sets of plates are prepared. The phages are adsorbed

on 112 Gal$^-$ cells and these cells spread on the first set. These are EMB galactose anti lambda serum plates. After 24 hours of incubation at 37°C. the colonies formed on these EMB plates are replicated (Lederberg and Lederberg, 1952) onto the two other sets. One of these sets is seeded with sensitive bacteria and after replication these plates are given a small dose of UV irradiation to induce the lysogenic cells. The other set has been prespread with either phage lambda or phage T4r. Both sets of replica plates are incubated at 37°C. On the set of plates seeded with sensitive bacteria the lysogenic colonies carrying a non-defective prophage are surrounded by a halo of lysis. On the lambda or T4r set these lysogenic cells form colonies since they are immune to lambda and since T4r does not multiply in K12 carrying the prophage lambda (Benzer, 1955). The defective lysogenic colonies (immunes) grow on the set of lambda or T4r plates but they give no halo on the set of sensitive bacteria plates. The sensitive colonies give no halo on the set of sensitive bacteria plates and do not grow on lambda or T4r plates.

Finally the transduced Gal$^+$ colonies can be recognised by their color on the EMB plates. This method permits a complete characterization of each type of colony to be determined. In all our experiments the transduced Gal$^+$ colonies invariably carried lambda (with or without defect).

Results:--Properties of the lambda HFT.--The phages obtained by induction of the primary heterogenote strain of bacteria have the same plaque morphology as the phages used to obtain the heterogenote.

Upon infection of sensitive Gal$^-$ cells with lambda HFT at multi-

plicities between 0.5 and 1 phage per bacterium it is found that between 5 and 15% of the colonies formed by the infected cells are either Gal$^+$ or form Gal$^+$ papillae and that each of these colonies is either lysogenic or immune. In addition another 5 to 15% of the colonies are lysogenic without being Gal$^+$. The remainder of the infected cells lyse or are refractor (Lieb, 1953). Thus in a lysate containing $10^{10}$ phages per ml., obtained after induction and lysis of $10^8$ cells per ml. at least 15% of the phages are able to transduce Gal$^+$. Thus at least 15 times more transducing phages are found than there were cells that liberated these phages. Even if each bacterium contained 4 prophages and 4 Gal$^+$ markers one would have to conclude that after induction the Gal$^+$ marker had multiplied 4 times. This is a lower limit for two reasons. First the phages which elicited a lytic response in the infected bacteria contained presumably a similar proportion of Gal$^+$ carriers as do the lysogenizing phages, second the failure of a lysogenizing phage to transduce the Gal$^+$ marker does not prove that the marker is absent from the phage.

Any cell transduced by lambda HFT is a heterogenote: upon multiplying they all segregate Gal$^-$ cells, all are lysogenic (non defective or defective) and the phages liberated by the lysogenic strains after induction transduce with a very high efficiency.

When lambda HFT infects <u>lysogenic</u> 112 Gal$^-$ cells its efficiency of transduction is about ten times smaller (per adsorbed phage) than when it infects sensitive cells. When the phage carried by the Gal$^-$ cells is different genetically from the phage HFT it is found that a very large proportion of the Gal$^+$ transduced cells still carry the phage for which they were originally lysogenic. (A small number of doubly lysogenic

cells and of immune cells are also found.) These transduced cells give heterogenote strains and the phages they liberate after induction are HFT. In these conditions it seems that only the Gal$^+$ marker has been added to the genome of the lysogenic cells while the genome proper of the transducing phages has been eliminated.

Lytic cycle of lambda HFT in a sensitive cell.--The multiplication of the Gal$^+$ marker which seems to take place in heterogenote cells after induction might be due to the special nature of these cells or to the UV induction proper. To probe further into the tie-up between phage multiplication and Gal$^+$ marker multiplication is secondary, HFT phages, grown for one cycle on sensitive cells, were studied. To compare the transducing ability of primary and secondary HFT lysates (equal aliquots of) 112 Gal$^-$ sensitive cells were infected with the same multiplicity of the two phage stocks (usually about 0.1 phage/bacterium) and spread on EMB galactose antiserum plates. The ratio of counts of transduced colonies on these plates is a direct measure of the relative efficiencies of transduction of the two sorts of phages.

The result of such measurements show that secondary HFT phages obtained by growth in C600 Gal$^+$ sensitive cells retain about 50% of their transducing ability per phage. Since in the growth experiments the average burst size is about 50 phages per bacterium, the Gal$^+$ marker has multiplied about 25 times. Thus on passing an HFT lysate through one cycle of growth on C600 Gal$^+$ sensitive cells the phage titer and the Gal$^+$ titer go up nearly in parallel. In contrast when secondary HFT phage is obtained by growth on 112 Gal$^-$ sensitive cells the lysate contains (for an average burst size of 50 phage per bacterium) about 1% of the original transducing ability per phage. In

other words on passing an HFT lysate through 112 Gal$^-$ sensitive cells the phage titer goes up but the Gal$^+$ titer remains constant. To insure that the failure of Gal$^+$ to multiply is not due to some special property of the strain 112 but is determined by the absence of the Gal$^+$ marker in the sensitive strains in which the growth of phage took place, a 112 Gal$^-$ sensitive was prepared as described in the following section. The colonies of this strain on EMB galactose plates have the same color as the colonies of 112 heterogenotes and thus are quite different from C600 Gal$^-$ colonies. When used as a host for HFT phages this Gal$^-$ strain gave the same results as those obtained with the host C600 Gal$^+$.

It thus seems that in secondary HFT the inclusion of the Gal$^+$ marker in the phage genome depends on the homologous marker present in the host bacterium.

Possible linkage between Gal$^-$ and the genetic markers of the HFT phages.--Bacterial recombination studies have shown that in a stable Gal$^-$ lysogenic strain the prophage is linked to the Gal$^+$ marker. The question arises as to the linkage between prophage and Gal$^-$ in the heterogenotes or in the HFT phages they liberate after induction.

This could be studied by the usual methods of phage genetics superinfecting the induced heterogenote with a genetically marked phage and looking at the markers carried by the transducing phages of the lysate. The lysate would then contain a large proportion of parental HFT phages. These parental HFT can be eliminated by using an immune heterogenote. When such a strain is induced no parental phages are produced because of the defect carried by the prophage (Appleyard, 1954; Jacob and Wollman, 1956); only the recombinants between the superinfecting

phage and the prophage could be expected to contain HFT phages. Their detection would thus be made much easier. Thus in the experiments to be described an immune heterogenote, obtained by infection of 112 Gal$^-$ sensitive cells by lambda (+) HFT, was used. [This immune heterogenote strain segregates Gal$^+$ and Gal$^-$ sensitive stable cells and Gal$^-$ immunes which in turn segregate Gal$^-$ sensitive stable cells. It is one of the Gal$^+$ sensitive stable strains which was used in the experiments of the preceding section].

After induction of the immune heterogenote the cells lyse with a latent period of about one hour but produce no phage (for $10^9$ induced cells).

The genetic characters of the defective prophage can be studied by superinfection of the induced immune heterogenote with a marked phage, say lambda (ab). The lysate is found to contain about 5% of recombinants of the types (a+) (+b) and (++). If such a lysate is tested for transducing ability by infection of 112 Gal$^-$ sensitive cells it is found that the number of transducing particles is of the order of magnitude to the number of immune heterogenote induced superinfected cells. Unexpectedly, the vast majority of the transduced cells are immune (a small proportion of the transduced cells were all doubly lysogenic). A few of the transduced immune cells have been tested by superinfection for the phage markers carried. They were all (++).

Hence the Gal$^+$ marker has a tendency to remain associated with the markers of the prophage (++ and defect) of the cells which carried it, and does not seem to attach itself to the superinfecting phage.

## Discussion

Let us begin by recapitulating what has been found. Our starting material are several strains of bacteria, all of them derivatives of coli K12. The original coli K12 is lysogenic and the sensitive derivatives among our strains were obtained by previous workers by various treatments involving specifically, UV irradiation. Our lysogenic starting material was obtained b lysogenising one of these sensitive derivatives.

In confirmation, and partly in extension of the findings of Morse et al., we find that phage produced by the lysogenic strain transduces the $Gal^+$ marker of the donor to the sensitive $Gal^-$ acceptor, with the very low efficiency characteristic of other transduction systems. It turns out, however, that the transduced have not been transduced in the usual manner, involving substitution of a piece of donor genome, for a piece of acceptor genome. In the present case the transduced cells give rise to highly abnormal strains, called 'heterogenotes' by Morse et al. In the first place these strains are unstable with respect to the $Gal^+$ marker, they segregate stable $Gal^-$ strains. This shows that the $Gal^+$ marker has not been substituted for the $Gal^-$ marker, but has somehow been added to the genome of the bacterium. As to where it has been added, we obtain hints from further observations. First of all, the transduction is invariably linked with lysogenization. This is in contrast to other transductions where the two phenomena seem to be relatively independent events. The strict linkage between transduction and lysogenisation makes one suspect that the $Gal^+$ marker is tied more intimately to the phage in this case. This inference is strongly supported by the finding that phage produced by the heterogenotes has a

very high and specific transducing ability for Gal$^+$. Indeed, as our experiments show, each donor bacterium produces many Gal$^+$ transducing phages. This suggests that the association between the Gal$^+$ marker and the phage persists not only during lysogenization but even during the vegetative multiplication of the phage in the heterogenote. The phages produced by the heterogenotes seem to be normal in other respects. They produce lytic and lysogenic responses in sensitive cells in the same proportion as normal lysates. The transductions which these phages accomplish show the same abnormalities as the original transductions, namely, the transduced strains are unstable with respect to Gal$^+$, and they are invariably lysogenic. An appreciable proportion of the lysogenics are defective lysogenics. Such defectives were not found among the primary heterogenotes, but were also not carefully looked for.

Up to this point, the results lead to postulate the following simple picture: phage lambda does not have the ability, like certain other phages, to serve as a vector for random pieces of the donor genome. It does, however, have the ability to tie the Gal$^+$ marker of the donor to its own genome, and carry it to an acceptor. The bond is so intimate, moreover, that the Gal$^+$ marker does not become detached from the phage in the acceptor, it remains in the acceptor only if the phage gets hooked into its prophage position. Even more strikingly is this bond evidenced by the fact that the Gal$^+$ marker multiplies with the phage after induction of the heterogenote. That the bond is not absolutely tight is evidenced by the fact that only about 50% of the cells lysogenized by an heterogenote lysate are transduced. Our

experiments do not tell whether in the cases where transduction fails the Gal$^+$ marker got detached from the phage in the donor, in the free-phage state, or during lysogenization of the acceptor.

We have attempted to test the persistence of the association between the phage and the Gal$^+$ marker during the growth of the phage in sensitive strains. In the case where these sensitive strains were Gal$^+$ strains, our results are in agreement with the general picture developed up to this point: one finds that the Gal$^+$ marker multiplies with the phage, but not perfectly. When the phage increases 100-fold, the transducing titer increases only 50-fold. In the one case at our disposal in which the sensitive strain was a Gal$^-$ strain, we obtained the astonishing result that the phage titer went up, as usual, while the Gal$^+$ transducing titer remained constant. This is an unexpected result. We are inclined to let it stand as an isolated fact without trying to force it into our picture, until more related experimental material has been accumulated.

There are several more pieces of evidence which testify in favor of the linkage of the Gal$^+$ marker in the heterogenotes with the phage. When a lysogenic cell is infected with a phage, similar to its prophage it shows immunity. It is known that this immunity is not due to failure of adsorption of the infecting phage. Several lines of evidence indicate on the contrary that the phage enters the lysogenic cell but is unable to multiply or to lysogenize. Our experiments in which the infecting phage has attached to its genome the Gal$^+$ marker show that in this situation the Gal$^+$ marker can be transduced, though about 100 times more rarely than in the case of sensitive acceptors. The transduced cells are heterogenotes and lysogenic, and this we interpret to

mean again that the Gal$^+$ marker is attached to the prophage. The prophage which these heterogenotes carry is the one they carried before the infection, and not that which brought the Gal$^+$ marker. This is reasonable in view of the fact that prophage substitution is a rare event. We learn from this experiment that the Gal$^+$ marker of the incoming phage can switch position, from its linkage to the vector to an analogous linkage with the prophage in the acceptor.

The experiments on defective lysogenic heterogenotes strengthen our picture. Such strains lack the ability to produce mature phages after induction. This lack, however, can be circumvented by superinfection with a related phage. The superinfecting phage recombines genetically with the defective phages and also allows these phages to mature (probably by phenotypic mixing). The lysates thus produced contain a mixture of phages, those having the genotype of the superinfecting phage, a small proportion having recombinant genotypes and those having the genotype of the defective prophage (including the defect which behaves as a genetic marker). These last phages, when infecting sensitive bacteria, cannot produce mature progeny because of their defect, but they can lysogenize the cells and make defective lysogenic bacteria (and hence their presence in the lysate may be detected). The great majority of the cells transduced by the lysate of a superinfected induced defective heterogenote carry the defect and the other genetic markers of the prophage and not the markers of the superinfecting phage (introduced to enable the defective prophage to mature). Hence our experiments show a strong linkage of the Gal$^+$ marker with its defective prophage in the defective heterogenote.

Summary.--

Phages obtained by induction of strain C600 Gal$^+$ made lysogenic for lambda ( ) when infecting strain 112 Gal$^-$ sensitive transduce Gal with low frequency. The strain C600 Gal$^+$ ( ) is stable, it does not segregate Gal$^-$ or immunes or sensitives. The transduced strains are Heterogenotes." These heterogenotes have the following properties:

1. They are Gal$^+$ and lysogenic.
2. They segregate Gal$^-$ lysogenic.
3. They do not segregate defective lysogenic or sensitives.
4. They produce primary HFT lysates after induction.

These primary HFT lysates have the following properties:

1. The burst size is about 100 phages per bacterium.
2. They produce lytic or lysogenic responses in sensitive cells in the same proportion as normal lysates.
3. Among the lysogenized cells there are a large number which have been transduced to Gal$^+$.
4. All the Gal$^+$ are lysogenic (Many carry defectives prophages).
5. The Gal$^+$ titer of the lysate is much higher than the titer of the induced bacteria which produced the lysate.
6. When the primary HFT lysate is passed through one cycle of growth on C600 Gal$^-$ sensitive or on 112 Gal$^-$ sensitive the phage titer and the Gal$^+$ titer go up nearly in parallel.
7. When the HFT lysate is passed through one cycle of growth on 112 Gal$^-$ sensitive the phage titer increases but the Gal$^+$ titer remains constant.

8. The HFT lysate transduce 112 Gal$^-$ lysogenic cells with a much lower efficiency than they transduce sensitive cells.

9. All these transduced lysogenic cells are hterogenotes and the vast majority of them liberate upon induction HFT lysates whose phages do not have the genetic markers of the phages used to transduce the lysogenic cells but have the genetic markers of the prophage carried by these cells.

10. The defective heterogenotes when superinfected after induction give lysates with transducing ability and the vast majority of the transduced cells are defective and the phage has the genetic markers not of the superinfecting phage but of the phage carried by the original defective heterogenote.

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